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Viral Infections of the Fetus

DAVID T. IMAGAWA, PH D:* Knowledge pertaining to virus infection of the human fetus is relatively meager. There was a time when viral infections during pregnancy were regarded as the principal cause of congenital malformation. This concept has not been substantiated, for in many instances newer modern virological techniques have failed to incriminate viruses as causing congenital malformations. It has been known for many years that certain virus infections during pregnancy might cause fetal death or stillbirth, but except for cytomegaloviruses and rubella, there is no conclusive evidence that other viruses are important causes of congenital malformation in humans.

Much remains to be learned about the important problem of viral infections of the fetus. The present report summarizes some of the pertinent information currently available. An overview of the problem will be discussed first, followed by comments concerning the biological sequelae in the human fetus, and then methods for prevention and control of fetal infections. Finally, laboratory animal experiments, which may lead to better understanding of the disease processes by which viral agents produce malformation or abnormal function will be described.

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An Overview

MILAN FIALA, MD:† The role of intrauterine viral infections in causing congenital malformations has been previously defined so far only with cytomegalovirus[1,2] and rubella[3,4] although herpes simplex, mumps, influenza, varicella, vaccinia, Coxsackie B, and other viruses (either wild or attenuated vaccine strains) may be surmised to play a role in isolated cases. The incidence of virus infections in pregnancy is affected by the communicability of a particular virus, by the environmental situation and by humoral and cellular defenses of the mother. Several incompletely understood factors influence the transmission of virus from the mother to the fetus and the development of congenital malformations:

- The timing of infection during pregnancy (with rubella the maximum risk of heart and ocular abnormalities is present during the first two months).
- Absence of maternal immunity (the presence of any maternal hemagglutination-inhibiting, neutralizing or complement-fixing antibody protects

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the fetus from rubella; the role of maternal cytomegalovirus antibody in protection of the fetus is not clear at present, although it has been proposed that cytomegalic inclusion disease occurs only when cytomegalovirus infection is primary[5] but not when it represents reactivation of a latent cervical infection of the mother.[6]

- Barrier function of the placenta.
- Immunological competence of fetus.

The viruses known or suspected to cause congenital abnormalities belong to different groups with widely different characteristics, including type of nucleic acid, structure and composition of virus capsid and interactions with host cells which range from mitogenic inhibition with rubella to lytic infection with Coxsackie B viruses.

Unlike cytomegalovirus infection, fetal infection with rubella virus often has severe consequences. The range of malformations due to rubella is very wide and probably due to the propensity of rubella virus to inhibit mitotic cell division. Major manifestations[7] include congenital heart disease, hearing loss due to damage to the inner ear, retinopathy and cataract (well known since the pioneering work of Gregg on the incidence of congenital blindness in relation to rubella epidemics[8]). During the neonatal period thrombocytopenic purpura or hepatitis and, later on, psychomotor retardation may develop.

In congenital cytomegalic inclusion disease—the most severe form of cytomegalovirus infection of fetus, probably acquired during the first 20 weeks of pregnancy—the most frequent congenital abnormality is microcephaly but other forms of neural and possibly extraneural (first arch and heart) abnormalities also occur. Most cytomegalovirus infections of the fetus are, however, asymptomatic or subtle, manifested by progressive developmental and mental retardation. Hepatosplenomegaly or hepatitis may be found at birth or develop postnatally.

Clinical and laboratory findings such as microcephaly, chorioretinitis and periventricular calcifications may suggest cytomegalovirus involvement; "pepper and salt" fundi or patent ductus arteriosus strongly point to congenital rubella. However, because of the similarity of clinical manifestations within the "TORCH" (toxoplasma, other viruses and syphilis, rubella, cytomegalovirus and herpes simplex virus) complex, specific diagnosis must be made by laboratory methods:

1. Virus isolation—virus infection may be chronic with prolonged virus excretion.

2. Antibody response—

(a) In the newborn: elevated IgM-globulin levels ($\geq$18 mg per 100 ml) are suggestive of a congenital infection.[9] Detection of specific IgM antibody to cytomegalovirus,[10] rubella virus, or toxoplasma in cord blood are considered as conclusive evidence for the role of each agent.

(b) Postnatally: in congenitally infected children specific IgG antibodies are rising while in children who acquired antibody passively from the mother the antibody titers are falling.

3. Histopathologic techniques—presence of large cells with intranuclear inclusions are characteristic of cytomegalovirus infection.

Biological Disturbances

JOSEPH W. ST. GEME, JR., MD:* Ordinarily one thinks of anatomical defects and embryogenetic anomalies when considering the impact of intrauterine viral infections in man. However, the physiologic and biologic sequelae of fetal viral infections are important, are poorly understood, and are more subtle than are gross, structural abnormalities. The pathogenetic mechanisms of the structural and biological abnormalities in the fetus include vascular, cellular, and placental factors. For example, rubella virus presumably replicates in the endothelial cells of the major and minor vasculature, inducing vascular injury and impairment of blood flow to differentiating and developing tissues.[11] The nucleic acid and protein metabolism of infected cells may be altered by some viruses with resultant diminution of cellular replication and possibly function.[12,13] If gestational viral infections modify either the development or the anatomic integrity of the placental vasculature, as well as the constituency and biochemical function of the intrinsic cellular population of the placenta, one could envision a profound impact on placental function and consequent fetal development.

An epidemiologic perspective of potential fetal viral infections is important. By no means are maternal infections with the two major viral pathogens rare. The incidence of rubella during pregnancy is ordinarily about 0.1 percent.[14] During the 1964-1965 epidemic the incidence was 2 percent. Approximately 5 percent of women develop rising titers of cytomegalovirus antibody during pregnancy and an astonishing 1 percent of newly born infants shed the virus in their urine.[14,15] The major

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source of perinatal cytomegalovirus infection may be third trimester transplacental maternal viremia, perhaps ascending transcervical infection, or the viral exposure of vaginal delivery, since 12 to 28 percent of pregnant women shed this virus in their cervical secretions during the late phase of gestation.[16,17] We suspect that the more frequent *late* fetal cytomegalovirus infection is also more benign than transplacental infection during *early* pregnancy.

Growth Retardation

One of the most striking sequelae of gestation viral infection is diminished fetal growth. Fetal growth retardation may be the only manifestation of congenital rubella virus and cytomegalovirus infection. Postnatal growth is also impaired, as is true for infants who have incurred fetal stunting for other reasons. Ultimately, in the absence of structural and functional abnormalities of the brain and heart, one might predict that the majority of these infants should attain nearly normal stature. Michaels and Kenny[18] made careful sequential observations of infants congenitally infected with rubella virus. They reported that these infants possessed no endocrine abnormalities and began to accelerate their growth when rubella virus disappeared from their tissues between six and twelve months of age. We need many more data of this sort. Several of the large centers which documented substantial numbers of young infants with the congenital rubella syndrome in 1964 may be able to provide information about subsequent growth, eight to ten years later.

The most crucial gauge of fetal and postnatal development is cerebral growth. Head circumference is also reduced by transplacental rubella virus and cytomegalovirus infection.[18-20] In some instances, profound microcephaly occurs, particularly in infants afflicted with more severe, generalized fetal cytomegalovirus infection. Not enough data are available to predict the rate of restoration of head circumference of mildly affected infants to normal dimensions during the early years of life.

Naeye[21,22] demonstrated that the diminished weight of fetal organs with congenital rubella virus and cytomegalovirus infections is due to decreased numbers of cells in the tissues rather than to a reduction in the size of cells. Unfortunately, available data are inadequate to determine whether the same is true for the placenta. Rawls and Melnick[13] cultivated the tissues of rubella virus-infected embryos and observed an impressive limitation in rates of cellular replication when compared with normal tissues. Furthermore, Plotkin and Vaheri[23] demonstrated an inhibitor substance elaborated by virus-infected cells which curbed the multiplication of normal cells. Although the presence of this inhibitor has not been substantiated by other investigators, it offers a logical explanation for the otherwise remarkable fact that the size of an organ may be decreased, yet only 0.1 percent of the cells contain rubella virus.[24]

It is conceivable that the vasculopathic components of gestational rubella virus infection exert a deleterious effect on the nutrition of embryonic and fetal tissues, with consequent attenuation of cellular division. The direct cellular and vascular aspects of placental infection may suffice to produce placental dysfunction. The relationship between placental insufficiency and fetal malnutrition is well known. Ordinarily, the disturbance of growth is limited to weight alone. Placental dysfunction is also associated with hypoglycemia and hypocalcemia. These two metabolic disturbances have not been described with fetal rubella virus and cytomegalovirus infections. Extrapolating from these other experiments of nature, one might conclude that the fetal growth retardation of these gestational viral infections cannot be attributed to a primary disturbance of placental function.

Neurologic Sequelae

The devastating neurologic effects of congenital rubella virus and cytomegalovirus infection are well known. Encephalitis, persistent replication of virus in the brain, intracranical calcifications, and severe microcephaly constitute the signal features of overt neural infection, frequently incompatible with survival. The more compelling concern is the potential intellectual aftermath of the more subtle cerebral involvement of relative microcephaly. A substantial percentage of children with microcephaly as the only apparent early manifestation of congenital cytomegalovirus infection are obviously retarded when evaluated carefully during the immediate preschool years.[2] However, one should not conclude that all children afflicted with intrauterine viral infection are doomed to a future of intellectual inadequacy. Children with congenital cytomegalovirus infection who appear normal at birth and at one year have been shown to possess intellectual competence similar to that of

their cultural peers at four years of age.[25] A recent study of a large number of children with congenital rubella, including some with prior fetal growth retardation, small stature, and microcephaly, disclosed a normal distribution of mental function.[26]

A fascinating, but tragic, element of the encephalopathy of transplacental rubella virus infection, recognized only very recently, is infantile autism.[27] This observation opens exciting new elements of speculation concerning this perplexing and serious behavioral disorder. Some children may have no other manifestations of transplacental rubella virus infection with this psychopathic condition.

Immunologic Deficiency

Profound maturational defects of immunoglobulin synthesis have been described with congenital rubella virus infection. Hypergammaglobulinemia and dysgammaglobulinemia have occurred in combination with the usual anatomic abnormalities.[28,29] More subtle immunobiologic perturbations have been detected by Michaels.[30,31] Rubella virus-infected infants are unable to produce the anticipated titers of antibody to the traditional immunizing biologicals of childhood. When rubella virus disappears from the pharynx and urine of these infants, the capacity to marshal an appropriate antibody response appears. There also exists a transient deficit in the expression of cell-mediated immunity or delayed hypersensitivity.[31-33] Since other natural and experimental viral infections shut off normal humoral and cellular immunity, it should not be surprising that a transplacental viral infection, with persistence of virus replication throughout several months of postnatal life, may also interfere with B-cell and T-cell function.[34] Although more data are required, infants congenitally infected with cytomegalovirus are adequately immunoresponsive to diphtheria toxoid.[31]

Altered Hematopoiesis

Because of the similarities between hematopoietic and immunopoietic cellular development, it is of more than passing interest that extramedullary hematopoiesis has been described in the skin of infants with congenital rubella virus and cytomegalovirus infection.[35] Inadequate immunocellular coping with persistent fetal virus infection may provoke a distorted proliferation of primitive stem cells as an alternate immunoresponsive cell population. It is intriguing that rare histocytic reactions and lesions have been described with both congenital rubella virus and cytomegalovirus infections.[36,37]

In 1941, Gregg[8] described the association of cataracts with congenital rubella. Soon thereafter, the sequelae of transplacental rubella virus infection were recognized as the classic tetrad of cataracts, patent ductus arteriosus, deafness and microcephaly. In 1964, the expanded rubella syndrome was delineated; it included, as newly or more clearly recognized manifestations, hepatosplenomegaly, thrombocytopenia purpura, metaphysitis, encephalitis, peripheral pulmonic artery stenosis, interstitial pneumonia, hepatitis, and intrauterine growth retardation.[4] Several years later, less obvious sequelae of fetal rubella virus infection emerged as clinicians began to look for distortion of physiologic function rather than merely anatomic defects. In the late 1950's, when the more definitive virologic and clinical studies of congenital cytomegalovirus infection were undertaken, the disease loomed as a rare, diffuse, devastating, and usually fatal process.[1] It is obvious now, almost 20 years later, that these early observations were focused only on the top of the pathophysiologic iceberg.

Proposed Solutions

TERRY YAMAUCHI, MD:* For the past three decades scientists have sought the best method to prevent, control, or treat viral infections of the human fetus. More productive investigation is needed for effective treatment of the maternal infection or the congenitally infected fetus. At present, the antiviral agents available make this course unfeasible. Drugs such as iodo-deoxyuridine and cytosine arabinoside effectively prevent replication of DNA viruses *in vitro*.[38] However, the very nature of their action, the inhibition of nucleic acid synthesis, precludes their use in a pregnant woman or fetus with actively dividing cell populations. An antiviral agent which will inhibit or destroy only the infecting virus and spare the normal host cells would be ideal.

Another method of controlling viral infections is early diagnosis of maternal infection followed by selective abortion of the exposed fetus. If a pregnant woman has an overt illness, such as rubella, the diagnosis is relatively easy. However, often there is only a history of a mild, non-specific illness, a cytomegalovirus infection, or recent

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exposure to rubella. In these cases the current techniques to establish the diagnosis of a specific viral infection are of primary importance. The techniques include: Recovery of virus in the gravid woman from blood, throat or urine, ideally from the blood to document a viremia; or serological studies demonstrating a change in specific antibody titer. Serological studies are imperative in the management of a pregnant woman recently exposed to rubella. The patient with pre-existing antibodies is less susceptible to infection than is one who is sero-negative. Amniocentesis and the development of assay systems to aid diagnosis of inborn errors of metabolism[39] support the hope that intrauterine viral infections might be evaluated in a similar fashion.

The most effective solution to the problem of viral infections of the human fetus is to prevent the primary maternal infection. Methods of preventing infection in pregnant women include isolation from infectious diseases and the improvement of maternal immunologic defense mechanisms. To isolate all pregnant women or women about to become pregnant would be impossible. The improvement of maternal immunity is a field of active research today. The use of viral vaccines in a widespread immunization program is a logical approach to improving maternal resistance to fetopathic viruses. Such a program has been initiated to control rubella virus infection. The problems of using live, attenuated viral vaccines are well illustrated by the controversial rubella vaccine.[40,41] The purpose of the rubella vaccine is to protect the unborn fetus from rubella virus infection and consequent deformities. At present, this vaccine is the only immunization that is given not to protect the recipient from disease, but to protect a fetus which may not be conceived for many years after the vaccine is administered. Clinical investigation has substantiated the effectiveness of this vaccine in preventing overt illness.[42,43] It is logical to believe that vaccinating the mother will protect the fetus from rubella infection. But this may not be true. While the vaccine protects the woman from overt disease, a few subclinical infections have been reported.[44] These subclinical cases may harbor and shed the wild rubella virus without the clinical manifestations of the disease. However, viremia and subsequent infection of the fetus has not yet been demonstrated in such persons.

The duration of immunity following rubella vaccination is another unknown quantity. Though it appears to be long-lasting, it has not been studied long enough to substantiate unwavering durability. Some clinicians are concerned with the possibility that girls being vaccinated today will develop low titers of rubella antibody in the future and become susceptible to re-infection. An even greater fear is the possibility that low antibody titer will mask rubella infection, setting the stage for an epidemic of subclinical infections with a population of girls approaching the child-bearing age.

The effects of live, attenuated vaccines on the human fetus are unknown. Rubella vaccine in particular is contraindicated in pregnancy because of its potential teratogenicity.[45] In some cases women who inadvertently received rubella vaccine during early gestation and lacked serum antibody to the virus had therapeutic abortions because of the possibility of delivering a congenitally deformed infant. Teratogenicity in humans, however, has never been shown with attenuated strains of viruses, although the viruses have been recovered from the cervix, placenta, and fetus.[41,46,47] In animal models, attenuated strains of viruses have been shown to induce congenital malformations (see next section on animal models).

One of the goals of massive immunization programs has been the development of herd immunity. Theory suggests that if enough people are "immune" to rubella via vaccination, the natural disease would not spread. The effectiveness of this theory has been questioned. Studies involving closed populations, such as military recruits and retarded children in institutions, revealed that when 60 to 90 percent of the population were immunized, the susceptible individuals within the group nevertheless had rubella during epidemic periods.[48,49] Therefore, in order for herd immunity to be protective, if possible at all, even larger numbers of people would have to be immunized.

In summary, the management of viral infections of the human fetus requires early diagnosis, development of safe antiviral chemotherapy and a comprehensive immunization program with effective vaccines.

Animal Models

DR. IMAGAWA: As was pointed out earlier, knowledge of viral infections of human fetus is meager but examples of congenital infections of viral origin in domestic and experimental animals are

expanding in number. Through work with these animal models, information leading to better understanding of the pathogenesis of virus infections which produce malformations is being acquired. Such information should be helpful in developing methods to prevent teratogenic viral infections in man.

Listed in Tables 1 and 2 are examples of congenital viral infections in domestic and experimental animals. Of particular interest in the animal model systems are the two attenuated viruses which are ordinarily used for immunizations of the animals. Hog-cholera virus, a RNA myxovirus, attenuated by passage in rabbits, is an effective immunogen in an adult animal, but can cause abnormal litters when given to a pregnant sow.[50] In animals receiving vaccine viremia develops and transplacental infection may occur. Piglets from mothers receiving the live vaccine have anomalies of the kidneys, nose and central nervous system. The importance of adequate immunity in the prevention of transplacental infection was recently described.[70] Transplacental infection did not occur in sows which had antibody titers of 1:2 or greater at the time of inoculation with the attenuated hog cholera virus. On the other hand, transplacental passage of the virus was demonstrated in sows that were previously vaccinated but lacked evidence of neutralizing antibodies. Furthermore, the authors strongly recommended that contact between susceptible pregnant sows and recently vaccinated animals should be avoided.

Similarly, attenuated blue-tongue virus of sheep, a RNA arbovirus, given to the pregnant ewe produces multiple malformations and brain hypoplasia of her lamb.[51] It is a depressing fact that attenuated viruses which are used for immunizations and produce no virulent effects in adult animals can be so deleterious to the fetus. It is with this fact in mind that stringent precautions are urged to make sure that live attenuated rubella virus or any other live vaccine virus is not administered, intentionally or by accident, to pregnant women.

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TABLE 1.—*Congenital Viral Infections in Domestic Animals*

Virus	*Host*	*Effects*	*References*
Attenuated hog cholera virus	Swine	Fetal death and multiple malformations	Sautter, J.H., et al.[50]
Attenuated blue-tongue virus	Sheep	Fetal death, multiple malformations, brain hypoplasia	Schultz, G. and DeLay, P.D.[51]
Bovine parainfluenza[3]	Bovine	Fetal death	Sattar, S.A., et al.[52]
Bovine viral diarrhea-mucosal disease virus	Bovine	Fetal death	Alafson, P., et al.[53]
Bovine rhinotracheitis	Bovine	Fetal death	Lukas, G.N., et al.[54]
Feline panleukopenia virus	Cat	Cerebellar hypoplasia	Kilham, L., et al.[55]
Feline herpesvirus	Cat	Fetal death	Hoover, E.A. and Griesemer, R.A.[56]
Equine rhinopneumonitis virus	Horse	Fetal death	Domock, W.V.[57]

TABLE 2.—*Congenital Viral Infections in Experimental Animals*

Virus	*Host*	*Effects*	*References*
Rubella	Rabbit	Transplacental infection	Belcourt, R.J.P., et al.[58]
		Growth retardation and malformations	Kono, R., et al.[59] London, W.T., et al.[60]
	Monkey	Transplacental infection	Parkman, P.D., et al.[61]
		Growth retardation and ocular changes	Delahunt, C.S. and Rieser, N.[62]
	Rat	Growth retardation, heart and ocular changes	Cotlier, E., et al.[63]
	Ferret	Cerebrovascular lesions	Rorke, L.B., et al.[64]
Mumps	Chick and monkey	Growth retardation, dysgammaglobulinemia and cardiomyopathy	St. Geme. J.W., Jr. et al.[65,66,67]
Rat virus and minute virus of mice (picodna)	Rat, hamster, and mouse	Fetal death and cerebellar hypoplasia	Kilham, L. and Margolis, G.[68,69]

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